Contributions to the Biochemistry of Growth.—The Total Nitrogen Metabolism of Rats bearing Malignant New Growths.*

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The following experiments were carried out with the object (1) of studying the effect of a rapidly growing neoplasm on the metabolism of the normal animal which bears the tumour, with consideration also of the view that the new growth secretes, as has often been asserted, substances having a deleterious action on the tissues of the animal bearing the tumour; (2) of elucidating the processes determining the rapid proliferation of the cells of a malignant new growth, and the source of the nitrogenous material used by the tumour.

In order to obtain facts throwing light on these questions, we have determined the nitrogenous metabolism in three rats before and after implantation of a rapidly proliferating malignant new growth. So far as we are aware, no such experiments have been made before.

The tumour used for these experiments was a spindle-celled sarcoma of very rapid growth, obtained from the Imperial Cancer Research Fund (tumour J. R. S.). This tumour can be transplanted with a high percentage of success in young animals of 40 to 60 grammes weight; with older rats of over 100 grammes weight the percentage of progressively growing tumours is not so high, and preliminary transient growth occurs more frequently. Since young growing rats did not appear to be suitable for a metabolism experiment in which it was desirable to obtain a constant nitrogen output, we found it necessary to use older animals.

Three rats of about the same age and weight were kept on a constant diet of a uniform composition. The diet used consisted of 56 grammes of stale bread made into a pulp with 50 c.c. of milk. Of this pulp 40 grammes were given to each animal in two daily rations. When a constant nitrogen output had been obtained, the rats were inoculated with a measured dose (0·1 c.c.) of the tumour, and the observations continued for the succeeding 15 days. At the end of the first week tumours could be distinctly felt. On the 16th day after transplantation the rats were killed. One animal (Rat I) had developed

* This research is in continuation of paper in 'Roy. Soc. Proc.,' B, vol. 80, 1908, p. 263.

a large, progressively growing tumour (5·1 grammes). The second rat (Rat II) had a smaller tumour (weighing 1·55 grammes) which was not increasing in size, and which possibly would have undergone spontaneous absorption if the experiment had been continued. In the third animal (Rat III) only transient proliferation had taken place, and no tumour was found when the animal was killed. Rat III may therefore serve as a useful comparison while Rat I represents the conditions in an animal with a tumour following its normal course of progressive proliferation. The cages in which the animals were kept were similar to those used by Prof. Schäfer* in his experiments on feeding with pituitary substance. In these cages the urine and the fæces can be collected separately. The observations were made in periods of three days. The food was given in high, narrow beakers, fixed in the cage, a device which prevented the animals from spilling the food. The beakers were removed empty after one hour. The animals rapidly ate all the food given to them, and were in good condition throughout the experiment; but Rat III did not appear to be so lively during the last week of the experiment, and was not so hungry at the time of feeding as the others, so that in the last week the food had to be left in the cage during the whole day. We have repeatedly noticed that where this tumour is being absorbed after a rapid initial proliferation, the health of the animals appears to be affected.

The food was analysed at different times during the experiment. It was found to have a constant N percentage. Six duplicate analyses gave 1·08 grammes N as the amount of nitrogen given in the food (120 grammes) during each three-daily period.

The results obtained are given in Table I.

It will be seen that the three animals continued to retain nitrogen and to increase in weight after transplantation. During the first two or three periods after the transplantation, when the absolute amount of tumour growth is slight, and when the introduced cells are being provided with a new stroma, the nitrogen retention remained on a low level and the animals did not gain in weight. But when the tumour had once established itself and was growing rapidly (Rat I, periods from 8, VII to 13, VII), the nitrogen retention rose rapidly. It will be shown that this is due not only to the formation of new tumour tissue, but to the organism of the host itself retaining nitrogen and increasing in weight correspondingly. The absorption of food from the intestine, as indicated by the nitrogen of the fæces, remained about the same. The urine was examined for creatine during the first nine days after transplantation. No creatine was present.

* E. A. Schäfer, Croonian Lecture, "The Functions of the Pituitary Body," 'Roy. Soc. Proc.,' B, vol. 81, 1909, p. 442.

Table I.

Period.	Rat I. Urine. N in grms.	Rat I. Fæces. N in grms.	Rat I. N-retention in grms.	Rat I. Weight in grms.	Rat II. Urine. N in grms.	Rat II. Fæces. N in grms.	Rat II. N-retention in grms.	Rat II. Weight in grms.	Rat III. Urine. N in grms.	Rat III. Fæces. N in grms.	Rat III. N-retention in grms.	Rat III. Weight in grms.	N in food in grms.
19—21 VI	0·840	0·090	0·150	210	0·721	0·112	0·247	175	0·771	0·106	0·203	180	1·08
22—24 VI	0·847	0·081	0·152	220	0·706	0·125	0·249	190	0·741	0·096	0·243	195	1·08
25—27 VI	0·955	0·061	0·064	220	0·756	0·102	0·222	195	0·785	0·105	0·190	200	1·08
28 VI	TRANSPLANTATION.												
29 VI—1 VII	0·956	0·085	0·039	220	0·767	0·105	0·208	200	0·785	0·118	0·177	200	1·08
2—4 VII	0·890	0·102	0·088	220	0·805	0·066	0·209	200	0·801	0·096	0·183	200	1·08
5—7 VII	0·893	0·095	0·092	225	0·767	0·118	0·195	210	0·822	0·111	0·147	210	1·08
8—10 VII	0·813	0·071	0·196	235	0·759	0·120	0·201	210	0·808	0·118	0·154	210	1·08
11—13 VII	0·819	0·089	0·172	240	0·683	0·124	0·273	220	0·780	0·106	0·194	210	1·08
14 VII	.....	.....	Host 235 / Tumour 5		.....	.....	Host 218·5 / Tumour 1·5		.....	.....	No tumour.		

If the malignant new growth secreted a substance of the nature of a toxin, one would expect to find at least a diminished nitrogen retention, if not an actual loss of nitrogen as the tumour increases in size. Our experiments give no evidence of the presence of a substance having a disturbing action on the nitrogenous metabolism.

The nitrogen retained during the experiment must have been allotted partly to the somatic tissue of the host on the one hand and partly to the tumour on the other. The proportion of nitrogen which goes to build up the new growth was found by determining the nitrogen contained in the whole tumour. The results of the analyses of the tumours are given in Table II.

Table II.

	Amount of tumour used for analysis in grammes.	Nitrogen found in grammes.	Total weight of tumour in grammes.	Nitrogen in total tumour in grammes.	Average nitrogen-content of total tumour in grammes.
Rat I...	1·095	0·02534	5·10	0·1180	0·1220
	1·025	0·02534		0·1260	
Rat II...	0·297	0·0084	1·55	0·044	0·0435
	0·323	0·00896		0·043	

The proportion of N used to build up new somatic tissue can be found by subtracting the N-content of the tumour from the nitrogen retention of the host *plus* tumour.

Table III gives the proportions of nitrogen which have gone to the building up of the somatic tissue of the host and to the formation of tumour tissue respectively. The results can be stated best in the form of the quotient, N-retention : Increase in weight. This quotient indicates the nitrogen value of the unit of tumour tissue, and unit of somatic tissue of the host respectively.

It is obvious that, before transplantation, the relation N-retention to increase in weight should be the same in the three rats, and as a matter of fact this was found to be the case. Since metabolism experiments on rats have not often been made, we may point out here that the constancy of the figures obtained is evidence of the reliability of our observations.

The most striking result obtained after transplantation is the fact that the quotient is smaller in the case of the new growth than in the case of the somatic tissue of the host. In other words, less nitrogen is needed to build

Table III.

	Before transplantation (9 days).			After transplantation (15 days).		
	Increase in weight in grammes.	N-retention in grammes.	N-retention. Weight increase.	Increase in weight in grammes.	N-retention in grammes.	N-retention. Weight increase.
Rat I.....	10	0·37	0·037	20·1 (total) − 5·1 (tumour) 15·0 (host)	0·59 (total) −0·12 (tumour) 0·47 (host)	0·023 (tumour) 0·031 (host)
Rat II ...	20	0·72	0·036	25·0 (total) − 1·5 (tumour) 23·5 (host)	1·10 (total) −0·04 (tumour) 1·06 (host)	0·026 (tumour) 0·045 (host)
Rat III ...	20	0·64	0·032	10·0 (total) (No tumour)	0·85 (total) (No tumour)	0·085 (total) (No tumour)

up a given weight of tumour tissue than is necessary to build up an equal weight of somatic tissue. It is worth considering whether or not this result may, in itself, be an adequate explanation of the rapidity of growth of the tumour cells. The significance of this fact will be discussed in greater detail in a succeeding paper.

The question arises as to the source of the supply of nitrogenous material from which the tumour cells build up new tissue. We have seen already that in our experiments the tumour cells did not grow at the expense of the tissues of the host. They must therefore have elaborated the nitrogenous material taken in as food.

In a normal growing animal, part of the nitrogen of the food goes to repair the wear and tear which the cells have undergone; this fraction is represented by the nitrogen excretion of the animal in a state of nitrogen hunger. Another fraction is used for the building up of the growing tissues of the animal; this fraction is represented by the amount of nitrogen retained by the animal. A third fraction, which has no specific function and which can be replaced by fats or carbohydrates, serves as a source of energy; this fraction may vary within wide limits, and, together with the first-named fraction, it is represented by the amount of nitrogen excreted in the urine in an animal in a state of nitrogenous equilibrium. In the terminology of Rubner, these three fractions receive the name of "repair fraction" (Abnützungsquote), "growth fraction" (Wachstumsquote), and "ergogenic fraction" (dynamogener Verbrauch). It is obvious that a tumour growing in an animal does not derive its nitrogen from the repair fraction, since this

fraction must remain constant if the animal is to live, and since it will be increased rather by the presence of the tumour, which throws an extra strain on the organism of the host. The tumour cells must therefore obtain their nitrogen either from the growth fraction or from the ergogenic fraction.

In the first case, we would have to assume that the cells of the tumour have a higher affinity for nutritive material than the growing cells of the host, and that they withdraw some of the nitrogenous material which otherwise should have gone to the building up of new tissues of the host. One would then expect the growth of the host to be retarded, and there should be a gradual diminution in the amount of nitrogen retained by the host.

If, on the other hand, the tumour cells derive their nitrogen from the ergogenic fraction, it would be unnecessary to assume any difference between the affinity of the tumour cells for nutritive material and that of the growing cells of the host. We would have to conceive that, after the wear and tear of the organism of the host is replaced the growing cells, be they those of the host or those of the tumour, have a first call upon the remainder of the nitrogen, and that only the nitrogen which remains after their demands have been satisfied goes to furnish energy. In other words, there would be a sparing of the protein undergoing combustion in the organism of the host. In this case one would expect to find the retention of nitrogen by the host to remain constant and its weight to increase, while the nitrogen excretion in the urine would gradually diminish.

Table IV gives the nitrogen retention *per diem* in three different periods. The first period of the nine days before transplantation represents the normal condition. The second period of the first nine days after transplantation

Table IV.

Nitrogen-retention of host during different periods.

Period.	Rat I.		Rat II.		Rat III.	
	Total in grammes.	Per diem in grammes.	Total in grammes.	Per diem in grammes.	Total in grammes.	Per diem in grammes.
Before transplantation (9 days)	0·37	0·04	0·72	0·08	0·64	0·07
After transplantation, 1st period (9 days)	0·22	0·02	0·61	0·07	0·51	0·055
2nd period (6 days)	Total ... 0·37	—	Total ... 0·47	—	0·35	0·06
	—Tumour 0·12	—	—Tumour 0·04	—	No tumour	
	Host ... 0·25	0·04	Host ... 0·43	0·07		

represents the condition where the absolute amount of tumour growth was small, and where therefore practically all the nitrogen retained must have gone to the host. During this period new stroma is formed and the tumour establishes itself. During the third period, starting from the tenth day to the sixteenth after transplantation, the absolute amount of tumour growth was large, and practically all the nitrogen found in the tumour when the animals were killed was retained during this period. If this amount is subtracted from the total amount of nitrogen retained during the third period, one arrives at the amount of nitrogen retained by the host during that period.

Any changes in the metabolism appearing in the first period after transplantation can be attributed mainly to the processes following upon inoculation (stroma-formation, etc.), while in the second period after transplantation those changes which are due to the growth of the tumour cells should come out most clearly. We find from Table IV that during the second period after transplantation the nitrogen retention by the host remains as high as it was before transplantation. Table I shows that the nitrogen excretion in the urine gradually diminishes as the tumour grows. In a former paper by one of us* it has been shown that the slow growth of a tumour may have a favourable influence on the growth of the host, and certainly does not retard it. From what has been said above, it is clear that in our experiments the tumour cells derived their supply of nitrogenous material by absorbing a part of that fraction of the nitrogen which otherwise would have served as a source of energy. They do not compete with the growing cells of the host, but they add their demands to those of the growing somatic cells.

It is obvious that as the tumour increases in size the amount of nitrogen necessary to replace the wear and tear of the cells of the tumour-bearing animal increases. At the same time a much larger amount of nitrogen will be necessary to cover the demands of the growing tumour cells, which rapidly increase in number. The result will be that the ergogenic fraction gets smaller and smaller and eventually a condition will arise when the host is incapable of absorbing sufficient nutritive material to cover the metabolic expenses of the host. The animal will then be in a state of under-feeding. The study of this condition will no doubt yield interesting results, but their correct interpretation will present great difficulties, since the essential features of the growth of cancer will be either masked or complicated by secondary factors. This condition will be discussed in another paper. We have referred to it here only in order to emphasise the fact that in our present investigation we have avoided these conditions, and that our present

* Cramer, "The Gaseous Metabolism of Rats inoculated with Malignant New Growths," 'Phird Scientific Report of the Imperial Cancer Research Fund,' 1908, p. 427.

observations and conclusions are intentionally restricted to animals bearing tumours large enough to reveal any specific property or function of which the cells of a malignant new growth may be possessed, but not so large as to introduce secondary factors due merely to the excessive size of the tumours.

One other point must be noted, namely, the relation of the nitrogen retention to increase in weight of the somatic tissue of the host after transplantation (see Table III). In the case of the animal bearing a rapidly growing tumour (Rat I), this relation is the same after transplantation as it was before transplantation. In Rat III, however, when the initial proliferation had been followed by absorption, a remarkable change has taken place: the quotient is more than twice as high after transplantation as it was before transplantation. In other words, nitrogen had been retained out of proportion to the increase in weight. It might be suggested that some of the tissues of this animal had been so changed in composition that they contained more nitrogen after transplantation. This explanation is not a very plausible one, and the analysis of the various tissues, which will be given in the succeeding paper, show that it cannot be maintained, since the nitrogen percentage of the various tissues of Rat III agrees with the nitrogen percentage of the tissues of Rat I and Rat II.

It is only possible to explain this phenomenon by the assumption that a formation of nitrogenous tissue has taken place, while at the same time non-nitrogenous tissue (fat or glycogen) has been used up. Whether this process stands in any relation to the absorption of tumour tissue and the effects produced by such an absorption (immunisation), will have to be determined by further investigations. The marked constitutional changes which accompany and follow the absorption of a tumour leave little doubt that the metabolism of the animal is deeply affected by this process. It is suggestive to find that in Rat II, where there was apparently a concomitant absorption of the tumour, a slight increase in the quotient is found.

Summary.

The main result of these experiments is to be found in the following facts:—

1. Less nitrogen is necessary to build up a certain weight of tumour tissue than is necessary to build up an equal weight of the somatic tissues of the host.

2. Animals bearing tumours maintain their positive nitrogen balance, and the nitrogen retention actually increases with the size of the tumour.

3. In our experiments the cells of the new growth derived their nitrogenous material necessary for the building up of new tissue by a sparing action on

the protein metabolism. The tumour cells do not proliferate at the expense of the tissues of the host, nor is there any evidence that they have a higher affinity for nutritive material than the growing cells of the host.

4. There is no evidence of the existence of substances secreted by the tumour disturbing the nitrogenous metabolism by means of a toxic action on the tissues of the host.

5. It is specially pointed out that these conclusions refer only to animals bearing tumours of sufficient size to warrant the assumption that they would reveal any specific property or function which may be possessed by the cells of a neoplasm. The effects which a large tumour must necessarily produce by virtue of its mere mass are not here considered.

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Contributions to the Biochemistry of Growth.—Distribution of Nitrogenous Substances in Tumour and Somatic Tissues.*

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In the preceding paper† a determination of the distribution of the nitrogen retained during a metabolism experiment showed that less nitrogen is needed to build up a given weight of tumour tissue than is necessary to build up an equal weight of the somatic tissues of the host. If this result is correct, it would follow that cancerous tissue should have a lower nitrogen percentage than the somatic tissues of the host.

We have therefore carried out nitrogen estimations of various tissues of Rats I, II, and III used in the experiments described in the preceding paper.

In order to make our results applicable to carcinomatous tumours, we examined the tissues of mice of about the same age, bearing a rapidly growing

* This research is in continuation of papers in 'Roy. Soc. Proc.,' B, vol. 80, 1908, p. 263, and this vol., p. 307, *supra*.

† W. Cramer and Harold Pringle, 'Roy. Soc. Proc.,' *supra*, p. 307.